AD-A275 145

VTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	1993	Reprint

4. TITLE AND SUBTITLE
(see title on reprint)

5. FUNDING NUMBERS
PE: NWED QAXM
WU: 00105

6. AUTHOR(S)
Gilman et al.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Armed Forces Radiobiology Research Institute
8901 Wisconsin Ave.
Bethesda, MD 20889-5603

8. PERFORMING ORGANIZATION REPORT NUMBER
SR93-28

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814-4799

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

14. SUBJECT TERMS	15. NUMBER OF PAGES
	5
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED		

CLASSIFIED BY:

DECLASSIFY ON:

Free Radical Biology & Medicine, Vol. 15, pp. 671–675, 1993
Printed in the USA.

0891-5849/93 $6.00 + .00

Brief Communication

EFFECT OF OXIDATIVE STRESS ON EXCITATORY AMINO ACID RELEASE BY CEREBRAL CORTICAL SYNAPTOSOMES

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(*Received* 23 *February* 1993; *Revised* 14 *April* 1993; *Accepted* 21 *April* 1993)

Abstract—Previous studies in our laboratory have suggested that an oxidation reaction is responsible for the actions of free radicals to decrease synaptic potentials. Recently we observed that free radicals both decreased depolarization-induced vesicular release and enhanced basal, nonvesicular release of the excitatory amino acid, [^{3}H]L-glutamate. In order to evaluate the contribution of oxidative reactions to this latter effect, we evaluated the actions of the oxidizing agent chloramine-T on synaptosomal release of excitatory amino acids, using [^{3}H]D-aspartate as the exogenous label. Basal and depolarization evoked [^{3}H]D-aspartate release were calcium-independent and nonvesicular. Chloramine-T pretreatment significantly increased basal release, while having no effect on high K^+-evoked release. These data suggest that an oxidative process can mimic the free radical increase of basal release, as well as the decrease in synaptic potentials. On the other hand, the calcium-independent-evoked release may involve a different mechanism. Our results demonstrate that under basal, nondepolarizing conditions, oxidative stress exerts an adverse effect on the presynaptic nerve terminal, resulting in an increased release of potentially damaging excitatory amino acid neurotransmitters.

Keywords—Chloramine-T, Oxidation, Free radical, Excitatory, Synaptosome, Aspartate

INTRODUCTION

Oxygen free radicals are generated as intermediate by-products of cellular metabolism and are normally maintained at low, nontoxic levels by the action of free radical scavengers and antioxidants.[1–3] However, increases in free radicals are associated with certain conditions, such as aging, ischemia, ionizing radiation or some degenerative neurological disorders.[1,4–9]

Exposure to free radicals modifies neuronal function. In hippocampal slices, we have observed decreased synaptic efficacy and impaired generation of spikes.[10,11] We hypothesized that the decrease in spike generation was associated with lipid peroxidation, while the decrease in synaptic transmission was related to oxidation of proteins.[11,12] In support of this was the observation that the oxidizing agent chloramine-T, which specifically oxidizes exposed cysteine and methionine residues of proteins,[13,14] produced a decrease in synaptic potentials in hippocampus in a manner similar to that observed with radiation and hydroxyl radical exposure.[12] In addition, chloramine-T did not produce lipid peroxidation in the hippocampal slice preparation (Pellmar and Lee, unpublished observations).

In a recent study, we found that, in addition to decreasing the calcium-dependent vesicular release of excitatory neurotransmitter from synaptosomes, free radicals enhanced the basal, noncalcium dependent release.[15] This action of free radicals is consistent with the increased excitatory amino acid release shown in both cerebellar and hippocampal slices under conditions of free radical generation.[16–18] Although the vesicular release of excitatory amino acids is considered of primary importance in normal synaptic transmission, the cytoplasmic pool of glutamate plays a major role in the dynamics of glutamate handling in the nervous system.[30] In order to further examine the contribution of an oxidative reaction to the free radical effects on the cytoplasmic pool of transmitter, we evaluated the effects of chloramine-T on synaptosomal

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release of excitatory amino acids using [^{3}H]D-aspartate which is not accumulated by the synaptic vesicles and, therefore, would only label the cytoplasmic pool of excitatory neurotransmitter.

MATERIALS AND METHOD

Animals

Adult male Hartley guinea pigs (200–400 g) were housed under a 12-h light–dark cycle. Commercial guinea pig chow and water was provided ad lib.

Materials

Radioactive [^{2,3,3}H]D-aspartate (specific activity 15–25 Ci/mM) was purchased from New England Nuclear (Boston MA). All other chemicals were obtained from Sigma Chemical (St. Louis, MO). All solutions were prepared with double distilled water.

Synaptosomal release studies

Guinea pigs were killed by cervical dislocation under isoflurane anesthesia. The brains were removed and cerebral cortices dissected out and placed in 10 volumes of ice-cold 0.32 M sucrose buffered with 5 mM TRIS, pH 7.4. Synaptosomes were prepared using a modification of the discontinuous density gradient centrifugation method of Dodd et al.,[19] as described previously.[20] The method used for radioactive amino acid loading of the synaptosomes was a modification of the method of Raiteri et al.[21] which has also been described previously.[22,23] Briefly, 1 ml aliquots (containing 2–4 mg protein/ml) of the synaptosome suspension were incubated at 37°C for 15 min to allow functional and metabolic equilibration. The synaptosome suspension (containing 2–4 mg protein/ml) was then loaded for 5 min with [^{2,3,3}H]D-aspartate. Amino acid neurotransmitter release determinations were made using a rapid superfusion technique described in previous studies from this laboratory.[15,24] Briefly, a 1.0 ml aliquot of the [^{3}H]D-aspartate-loaded synaptosome suspension was poured over a 0.45 micron nylon filter staged on a multiforated support of a 10 ml superfusion chamber. The chamber was connected to a peristalic pump and the filter washed for 15 min with a calcium-free 5 mM K^+ buffer solution. During this washout period, the perfusate consisted of buffer alone or buffer containing 0.1 mM chloramine-T. Following the washout period, a line from the peristalic pump was aligned directly over a scintillation vial. Two wash (W) perfusates, each containing 0.5 ml of perfusate, were collected into scintillation vials. Ten mililiters of a 5 mM K^+-containing, nondepolarizing, or a 55 mM K^+ depolarizing medium was poured into the superfusion chamber. Eight efflux fractions containing 0.5 ml each of perfusate were collected every 10 s into scintillation vials. Ten mililiters of Biofluor was added to each of the vials (2 W and 8 efflux) and radioactivity determined by liquid scintillation spectrometery. The filter was placed in a scintillation vial and the synaptosomes solubilized prior to counting. Fractional efflux of [^{3}H]D-aspartate was expressed as percentage of total radioactivity, where total radioactivity was the sum of all fractional perfusate dpm values and dpm remaining on the filter.

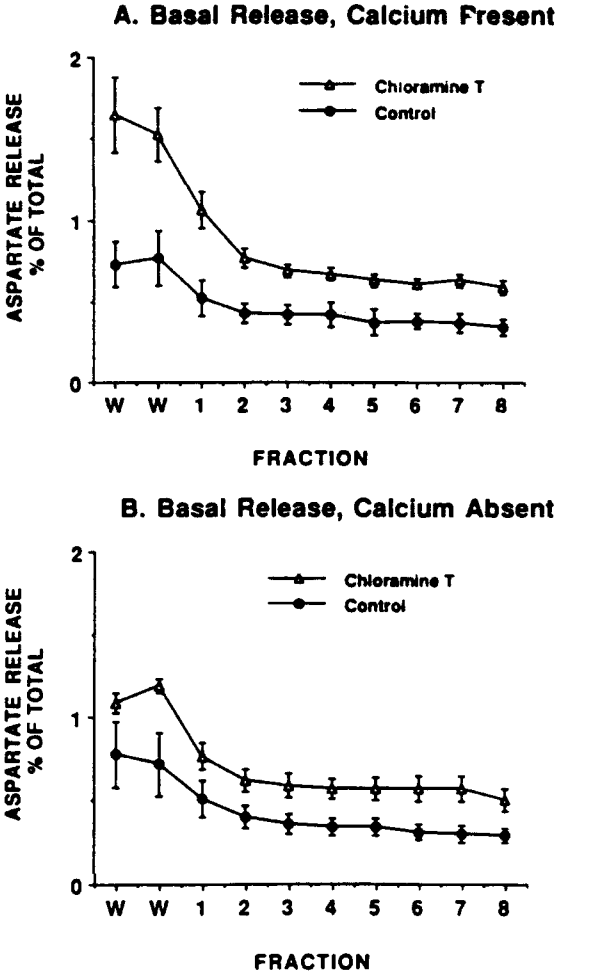

Fig. 1. Effect of 0.1 mM chloramine-T on spontaneous, nondepolarized (5 mM K^+-evoked) release of [^{3}H]D-aspartate. (A) In the presence of calcium. (B) In the absence of calcium. W represents wash perfusates as described in the Materials and Method section. Each point represents mean and SEM of eight experiments.

RESULTS

Pretreatment of synaptosome fractions with 0.1 mM of chloramine-T increased the basal release of [^{3}H]D-aspartate in the presence of calcium (Fig. 1A).

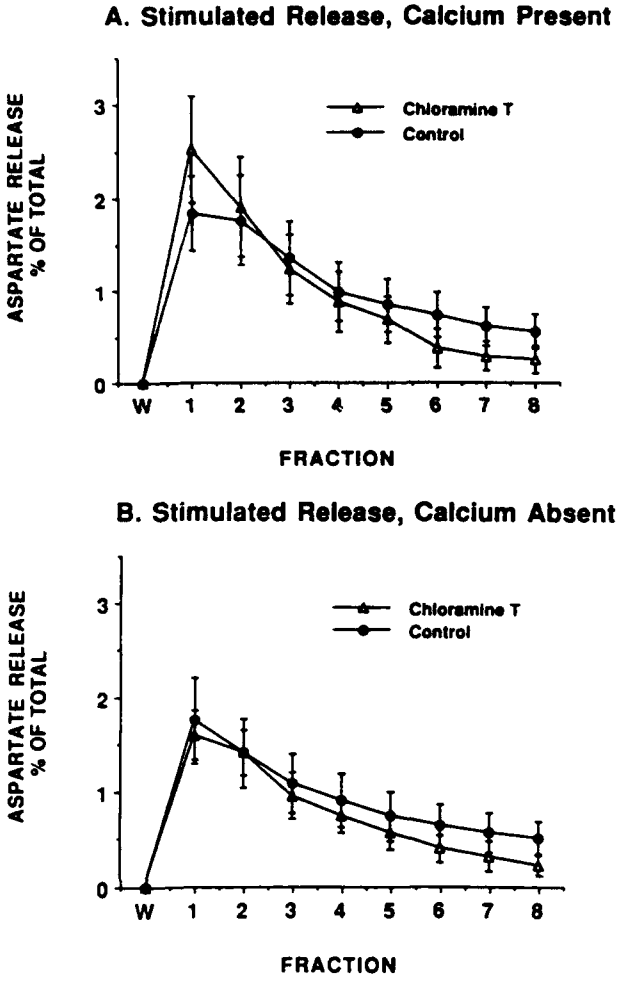

Fig. 2. Effect of chloramine-T on depolarized (high K^+-evoked) release of [^{3}H]D-aspartate from cerebral cortical synaptosomes. (A) In the presence of calcium. (B) In the absence of calcium. W represents wash perfusates as described in the Materials and Method section. Each point represents the mean and SEM of eight experiments.

Spontaneous release of [^{3}H]D-aspartate increased 1.5–2.0 fold over control values. The increase in nondepolarized release in the presence of calcium was significant at all time points ($p < .05$). Removal of calcium from the media did not affect the basal release of [^{3}H]D-aspartate (Fig. 1a, closed circles vs. Fig. 1b, closed circles). Chloramine-T was less effective in affecting basal [^{3}H]D-aspartate release when calcium was removed from the solution (Fig. 1A, open triangles vs. Fig. 1B, open triangles), but the increase remained significant at all time points.

Figure 2 illustrates the effect of chloramine-T on high K^+-evoked [^{3}H]D-aspartate release. Basal levels for each experiment were normalized by subtracting the value of the final wash (W_2) from all data points. As can be seen in Fig. 2A, the high K^+-evoked release of [^{3}H]D-aspartate from the cerebral cortical synaptosomes in the presence of calcium was not affected by 0.1 mM of chloramine-T. Removal of calcium had no effect on stimulated [^{3}H]D-aspartate release under control conditions (Fig. 2A, closed circles vs. Fig. 2B, closed circles) or in the presence of chloramine-T (Fig. 2A, open triangles vs. Fig. 2B, open triangles).

DISCUSSION

The present study demonstrates that chloramine-T can mimic the previously reported effects of peroxide-generated free radicals to enhance nonvesicular release of excitatory amino acid neurotransmitters. Use of [^{3}H]D-aspartate, the nonmetabolized analogue of L-glutamate and L-aspartate, which is not taken up by the presynaptic vesicles,[25–27] allowed us to target our investigation to release from the cytoplasmic pools of excitatory amino acids. As expected,[24,28,29] the actions of chloramine-T on both the basal and evoked release of [^{3}H]D-aspartate were calcium independent.

Basal release

Under normal conditions, basal release of excitatory amino acids is maintained at a low level, reflecting an equilibrium in the extracellular and intracellular distribution of the amino acids. A number of factors such as membrane integrity, membrane potential, ion concentration gradients, and amino acid transporters contribute to maintaining this concentration ratio.[30] The enhanced basal release observed with peroxide,[15] and with chloramine-T in the present study, could reflect changes in any of these processes. A general membrane disruption by chloramine-T is unlikely since the oxidant has no proteolytic activity.[31] In addition, chloramine-T has a very specific electrophysiological effect on synaptic efficacy without affecting the ability to generate spikes.[12] This would be an unlikely consequence of breakdown of membrane integrity. Nonspecific changes associated with membrane depolarization are also unlikely, since we have found that free radicals do not alter membrane potential in hippocampal neurons,[10] despite reported depolarization of cardiac myocytes.[32]

Specific impairment of an amino acid transporter is perhaps more feasible as a protein target for chloramine-T and free radicals. Neuronal reuptake of the excitatory amino acids depends primarily on a high affinity sodium/amino acid cotransporter.[30] Chloride-, hydrogen-, and calcium-dependent transport processes also contribute to the reuptake of excitatory amino acids. Any of these transport systems may be

targeted by the oxidant, resulting in a net loss of the amino acid from the nerve terminal.

Since the amino acid uptake systems are dependent on ionic gradients for the transport process, disruption of these gradients would also disrupt uptake. For example, chloramine-T is known to inhibit sodium current inactivation in some neuronal preparations.[33–35] This could increase intracellular sodium and reduce the sodium gradient primarily responsible for glutamate uptake. Changes in sodium-potassium or sodium-calcium exchange pumps similarly could modulate the handling of excitatory amino acids.

The enhanced basal release produced by chloramine-T is very similar to the changes observed with exposure to hydrogen peroxide.[15] Our results suggest that an oxidation of a cellular protein is responsible for the free radical effects on this process. An inhibition of glutamate uptake by superoxide has been recently described by Volterra et al.[36] A similar target for both peroxide-generated hydroxyl radicals and for chloramine-T is not unlikely.

Evoked release

As with basal release, the evoked efflux of [^{3}H]D-aspartate was calcium independent, as observed previously.[28,29,31] The mechanisms of calcium-independent evoked release of amino acid neurotransmitters are unknown.[30]

Many studies suggest the influx of sodium ions as the link between depolarization and release in the absence of calcium. Alternatives include many of the same mechanisms suggested for calcium-independent basal release. Our results show a differential effect of chloramine-T on basal and evoked release. While basal release was enhanced, evoked release was unaffected. These data would suggest that the two processes occur through distinct mechanisms and that only basal release involves a step that is sensitive to protein oxidation.

In conclusion, this study has shown that the oxidizing agent, chloramine-T can mimic free radical actions to enhance basal, nondepolarized release of excitatory amino acids. We previously showed that chloramine-T decreased synaptic potentials, presumably through an inhibition of vesicular neurotransmitter release. A protein oxidation reaction (as opposed to lipid peroxidation) is suggested as the mechanism for these presynaptic actions of free radicals.

Acknowledgement—This work was supported by the Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, under work unit 00105. The views presented in this paper are those of the authors; no endorsement by the Defense Nuclear Agency or the Department of Defense has been given or should be inferred. Research was conducted according to the principles enunciated in the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council.

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